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Effects of whole-stream nitrogen enrichment and litter species mixing on litter decomposition and associated fungi

Verónica Ferreira*, Manuel A.S. Graça

MARE-Marine and Environmental Sciences Centre, Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, 3004-517 Coimbra, Portugal

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ABSTRACT

Nutrient enrichment and changes in riparian tree species composition affect many streams worldwide but their combined effects on decomposers and litter decomposition have been rarely assessed. In this study we assessed the effects of experimental nitrogen (N) enrichment of a small forest stream on the decomposition of three leaf litter species differing in initial chemical composition [alder (*Alnus glutinosa*), chestnut (*Castanea sativa*) and poplar (*Populus nigra*)], incubated individually and in 2-species mixtures during late spring-early summer. To better understand the effects of litter mixing on litter decomposition, component litter species were processed individually for remaining mass and fungal reproductive activity. Litter decomposition rates were high. Nitrogen enrichment significantly stimulated litter decomposition only for alder incubated individually. Differences among litter treatments were found only at the N enriched site where the nutrient rich alder litter decomposed faster than all other litter treatments; only at this site was there a significant relationship between litter decomposition and initial litter N concentration. Decomposition rates of all litter mixtures were lower than those expected from the decomposition rates of the component litter species incubated individually, at the N enriched and reference sites, suggesting antagonistic effects of litter mixing. Conidial production by aquatic hyphomycetes for each sampling date was not affected by nutrient enrichment, litter species or mixing. Aquatic hyphomycetes species richness for each sampling date was higher at the N enriched site than at the reference site and higher for alder litter than for chestnut and poplar, but no effect of mixing was found. Aquatic hyphomycetes communities were structured by litter identity and to a lesser extent by N enrichment, with no effect of mixing. This study suggests that nutrient enrichment and litter quality may not have such strong effects on decomposers and litter decomposition in warmer seasons contrary to what has been reported for autumn-winter. Changes in the composition of the riparian vegetation may have unpredictable effects on litter decomposition independently of streams trophic state.

1. Introduction

Small forest streams make up most of the water courses in temperate catchments (Allan and Castillo, 2007). In these streams, the aquatic food web is greatly fuelled by the autumnal leaf litter input derived from the riparian vegetation (Wallace et al., 1997). Once in water, this organic matter is rapidly colonized by microbial decomposers, especially aquatic hyphomycetes, who mineralize it and convert it into biomass (mycelium and conidia) leading to substantial litter mass loss (Gulis and Suberkropp, 2003). By the activities of their external enzymes and build up of mycelium, aquatic hyphomycetes also increase litter palatability to invertebrate detritivores, establishing the link between organic matter and secondary production (Canhoto and Graça, 2008).

The community structure and performance of aquatic hyphomycetes and litter decomposition are affected by environmental changes (Ferreira et al., 2014; Canhoto et al., 2016; Ferreira and Voronina, 2016). In particular, moderate increases in dissolved nutrients concentration generally modify aquatic hyphomycetes community structure (Gulis and Suberkropp, 2003; Castela et al., 2008; Lima-Fernandes et al., 2015) and stimulate fungal activities (with reproductive activity being most sensitive) and litter decomposition (Suberkropp and Chauvet, 1995; Gulis and Suberkropp, 2003; Gulis et al., 2006; Woodward et al., 2012; Ferreira et al., 2015). Increased nutrients concentration presently affects many streams (Woodward et al., 2012) and generally results

* Corresponding author.
E-mail address: veronica@ci.uc.pt (V. Ferreira).

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from agriculture, urbanization and atmospheric nitrogen (N) deposition (Rockström et al., 2009). Given the predicted increase in human population over this century, and consequent increases in the needs for food, water, transport and housing, nutrient loads to streams are expected to increase (MEA, 2005). Thus, many more streams will likely be affected by nutrient enrichment in the future, with consequences for aquatic communities and litter decomposition.

The performance of aquatic hyphomycetes and litter decomposition also depend on litter characteristics, with soft, high quality (low carbon: nutrients ratio, low lignin concentration) litter being colonized and decomposed faster than hard, low quality litter (Gessner and Chauvet, 1994; Lecerf and Chauvet, 2008; Schindler and Gessner, 2009; Frainer et al., 2015). Aquatic hyphomycete community structure often also differs between litter species (Canhoto and Graça, 1996; Gulis, 2001; Ferreira et al., 2006). Thus, an interaction between litter species and dissolved nutrients concentration on microbial activity and litter decomposition is expected. Indeed, the effects of nutrient enrichment on litter decomposition are usually stronger for low quality litter where microbial activity is likely nutrient limited (Gulis and Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006). However, most studies have addressed the effects of nutrient enrichment on the decomposition of litter species incubated individually (but see Rosemond et al., 2010; Lima-Fernandes et al., 2015), despite the fact that litter usually make multi-species mixtures in streambeds (Swan and Palmer, 2004; Molinero and Pozo, 2006).

Forestry practices, pathogens, species invasions and climate changes may promote modifications in the composition of riparian forests with species replacement or loss, and the consequent alteration of the identity and/or number of litter species in streambeds (Graça et al., 2002; Kominoski et al., 2013). Many studies have shown that the interactions between component litter species in litter mixtures commonly lead to non-additive effects of mixing on litter decomposition and that this often depends on the identity of the component litter species (reviewed by Lecerf et al., 2011). Non-additive effects of litter mixing on litter decomposition may be due to selective feeding by detritivores on the more palatable litter, nutrient transfer between litter species by fungal mycelium that may reduce nutrient limitation of microbial activities in the nutrient poor litter or leaching of secondary compounds (e.g. polyphenols) that can inhibit fungal exoenzymes in the neighbor litter (Gessner et al., 2010).

Since nutrient enrichment of streams and changes in riparian vegetation composition are likely to occur simultaneously in the future (MEA, 2005), it is important to assess the effects of nutrient enrichment and leaf species mixing on litter decomposition and associated fungi if we want to better predict the response of stream ecosystems to environmental change. The two studies that have so far assessed the interaction between stream nutrient enrichment and litter species mixing on litter decomposition and associated biota have found non-additive effects of mixing under low nutrient conditions but not under nutrient enrichment in autumn-winter (Rosemond et al., 2010; Lima-Fernandes et al., 2015).

In this study we assessed the effects of experimental N enrichment of a small forest stream and litter species mixing on the decomposition of 2-species mixtures and of component litter species incubated individually, and reproductive activity of fungi associated with individual litter species. We predicted that (a) fungal activity and decomposition rates would be faster at the N enriched site than at the reference site, due to a stimulation of fungal activity by nutrient enrichment, (b) stimulation of fungal activity and litter decomposition by N enrichment would be stronger for the N poor (chestnut and poplar) than for the N rich litter species (alder), (c) at the reference site, the 2-species mixtures whose component species differ the most in initial N concentrations would decompose faster than predicted by the decomposition rate of the component species individually (non-additive effects), (d) at the N enriched site, the decomposition rate of the 2-species mixtures would not differ from that predicted by the decomposition rate of the component species individually, as mass loss of N poor litter would not be N limited (additive effects), and (e) aquatic hyphomycete community structure would be sensitive to nutrient concentration, litter species and mixing. Since component litter species in mixtures may respond in opposite ways to mixing and lead to apparent additive effects ('counterbalance hypothesis'; Schindler and Gessner, 2009), the response of component litter species to N enrichment and mixing was assessed.

Table 1
Water physical and chemical variables (mean ± SE) at sites R (reference) and N (nutrient enriched) during the litter decomposition experiment (9 June – 14 July, 2008). Comparisons between sites were done by *t*-tests and p values are shown.

Water variables	n	Site R	Site N	p
Temperature (°C) at ~9 am	4	12.55 ± 0.27	12.65 ± 0.31	0.816
Conductivity (μS/cm)	4	63.75 ± 0.32	65.03 ± 0.78	0.183
Alkalinity (mg $CaCO_3$/L)	4	17.75 ± 0.59	17.10 ± 0.26	0.354
pH	4	6.86 ± 0.04	7.10 ± 0.03	0.003
O_2 (mg/L)	4	10.12 ± 0.06	10.35 ± 0.03	0.027
NO_3-N (μg/L)	6	186.77 ± 49.88	1681.25 ± 162.76	<0.001
SRP (μg/L)	4	62.38 ± 1.64	60.72 ± 1.77	0.516

SRP, soluble reactive phosphorus.
NO_2 and NH_4 were below the detection limit (<100 μg/L and <50 μg/L, respectively).

2. Methods

2.1. Study stream and experimental N enrichment

The experiment took place in a first-order stream located at Margaraça Forest, a protected area with low human activity in Açor Mountain (Central Portugal; 40°13′N, 7°56′W, 600 m a.s.l.). The stream flows over schist bedrock, across a mixed deciduous forest dominated by chestnuts (*Castanea sativa* Mill.) and oaks (*Quercus robur* L.)(Paiva, 1981). The stream is N limited (7–197 μg NO_3-N/L, 43–216 μg PO_4-P/L; range over autumn 2003 and 2004) and has low discharge (0.7–3.0 L/s; range over autumn 2003 and 2004), which makes it suitable to study the effects of experimental N enrichment on litter decomposition (Ferreira et al., 2006). More information on the stream and surrounding forest can be found in Paiva (1981), Abelho and Graça (1998) and Ferreira et al. (2006).

Two sites with similar characteristics were selected within the stream, with the upstream site acting as a reference (site R) and the downstream site being experimentally N enriched (site N) (Table 1). The study reach was ~1.5 m wide, ~10 cm deep and had a discharge of 4.4 ± 1.5 L/s (mean ± SE); sites were apart by ~3 m that coincided with a pronounced discontinuity in slope (abrupt fall by ~50 cm). Experimental N enrichment at site N began one month before the start of the litter decomposition experiment and was carried out continuously until the experiment ended by using $CaNO_3$ concentrated solution (550 g/L), dripping from ten 5-L glass Mariotte bottles (Fig. A1 in Appendix A). Bottles were refilled weekly and dripping rates set according with discharge, measured by chloride release (see below). This resulted in a ~9 fold increase in N concentration at site N over the reference concentration (Table 1). N concentration at site N was within the values found for streams affected by agricultural activities (Gulis et al., 2006).

2.2. Water variables

Conductivity, water temperature (WTW LF 330, WTW, Weilheim, Germany), dissolved oxygen concentration (WTW OXI 92, WTW, Weilheim, Germany) and pH (pH 3110, WTW, Weilheim,

Table 2
Initial chemical characterization (mean ± SE, n = 3) of the leaf litter used in the litter decomposition experiment. Comparisons among species were done by 1-way ANOVAs and p values are shown; different letters indicate significant differences (Tukey's test, $p < 0.050$).

Litter variables	Alder	Chestnut	Poplar	p
Phosphorus (% DM)	$0.10^{a} \pm 0.01$	$0.14^{a} \pm 0.02$	$0.10^{a} \pm 0.01$	0.146
Nitrogen (% DM)	$2.19^{a} \pm 0.15$	$1.05^{b} \pm 0.06$	$0.71^{b} \pm 0.04$	<0.001
Polyphenols (% DM)	$6.62^{a} \pm 0.25$	$10.93^{b} \pm 1.03$	$14.53^{c} \pm 0.73$	0.001
Lignin (% DM)	$28.61^{a} \pm 0.59$	$33.05^{a} \pm 0.66$	$46.47^{b} \pm 1.83$	<0.001

Germany) were recorded weekly at both stream sites with field probes. Additionally, 300 mL of stream water were filtered through glass fibre filters (47 mm diameter, pore size 0.7 μm; Millipore APFF04700, Millipore Corp., Billerica, MA, USA) at each site, stored on ice, and returned to the laboratory for the determination of NO_2, NO_3, NH_4 (ion chromatography, Dionex DX-120, Sunnyvale, CA, USA), soluble reactive phosphorus (SRP; ascorbic acid method) and alkalinity (titration with 0.2 N H_2SO_4 to a final pH of 4.2)(APHA, 1995). Discharge was estimated after chloride release (NaCl concentrated solution, 250 g/L), by the following formula: $D = ([Cl] \times \text{release rate})/((Cond_f - Cond_b) \times 0.32 + (-0.15))$, where D is the discharge in L/s, [Cl] is the Cl concentration in mg/L (=151709), release rate is the release rate of NaCl solution in L/s, $Cond_f$ is the conductivity after Cl concentration reached a plateau in stream water in μS/cm, $Cond_b$ is the background conductivity in μS/cm, 0.32 is the slope in the conductivity-chloride concentration linear regression equation and −0.15 is the intercept in the same equation (Mulholand et al., 1994; Webster and Ehrman, 1996).

2.3. Litter decomposition

Alder (A; *Alnus glutinosa* (L.) Gaertner) and poplar (P; *Populus nigra* L.) leaves were collected after senescence from single tree stands near Coimbra, Central Portugal, and chestnut (C; *C. sativa*) leaves were collected after senescence at Margaraça Forest, in autumn 2007. All three species are commonly present in the riparian forest of streams in Central Portugal. Leaf litter was stored in paper boxes and kept at room temperature in the dark until used. Before being used in the decomposition experiment, litter was characterized regarding initial N (acid digestion using $CuSO_4$ and H_2SO_4; Graça et al., 2005), phosphorus (acid digestion using HCl; Graça et al., 2005), polyphenols (Folin-Ciocalteu method; Graça et al., 2005) and lignin concentration (Goering and Van Soest method; Goering and Van Soest, 1970). These leaf species were chosen based on their initial N concentration (Table 2), so that the resulting 2-species mixtures included two species with contrasting initial N concentrations (AC and AP) and two species with similar N concentrations (CP).

Air dried leaves were enclosed in 15 × 20 cm coarse mesh bags (10-mm mesh opening), individually and in 2-species mixtures (six litter treatments in total: A, C, P, AC, AP and CP). Single species litter bags were prepared with 2.00 g (±0.01) of leaves, while 2-species litter bags included 1.00 g (±0.01) of each component leaf species. Fifteen litter bags from each litter treatment were anchored to the stream bed with nails, at both sites (180 bags total, 15 bags×6 treatments×2 sites), on 9 June 2008. The decomposition experiment took place in late spring-early summer, as warmer water temperature emphasizes diversity effects in litter mixtures (Swan and Palmer, 2004; Lecerf et al., 2011), which, accordingly to our predictions, are expected to occur at site R but not at site N. Litter decomposition assumes the strongest importance in fueling aquatic food webs with carbon and nutrients during autumn-winter as litter availability is highest during and following the autumnal litter fall in streams flowing through temperate deciduous forests (Molinero and Pozo, 2004). However, in streams receiving high inputs of medium-slow decomposing litter, this can remain an available resource throughout the year (Abelho and Graça, 1998; Molinero and Pozo, 2004). Thus, assessing decomposers activity during spring-summer is highly relevant to better understand ecosystem responses to environmental change.

Litter bags (3–6 replicates per treatment) were retrieved from each site weekly over 35 days (five sampling dates) for treatments without alder and over 28 days (four sampling dates) for treatments with alder due to fast decomposition of alder litter. Litter bags were enclosed in individual zip lock bags, stored on ice and returned to the laboratory where they were promptly processed. Component litter species from 2-species litter mixtures were processed individually. Litter was gently rinsed with distilled water on top of a 500 μm mesh sieve to retain small litter fragments and five discs (12 mm diameter) were cut out with a cork borer from individual leaves when possible to induce conidial production by aquatic hyphomycetes (see below). Remaining mass was oven-dried at 105 °C for 24 h and weighed (±0.1 mg) to determine dry mass (DM). The fraction of DM remaining, taking into account the discs removed (see below), was estimated as DM at the sampling date/initial DM. Initial DM was estimated from initial air-dry mass by applying a conversion factor. The air-dry mass to DM conversion factor was obtained from an extra set of three replicates of each litter treatment that was incubated in the stream for 10 min on day 0. These bags were returned to the laboratory and DM was determined as described above.

2.4. Conidial production by aquatic hyphomycetes

Conidial production (reproductive activity) by aquatic hyphomycetes was determined as this is a reliable indicator of fungal activity and one of the most sensitive variables to stream nutrient enrichment (Suberkropp, 1991; Gessner and Chauvet, 1994; Gulis and Suberkropp, 2003; Gulis et al., 2006). When remaining mass was enough, five litter discs were cut out to induce conidial production by aquatic hyphomycetes (Graça et al., 2005). Component species from litter mixtures were processed individually. Sporulation by aquatic hyphomycetes was induced by placing the litter discs in 100 mL Erlenmeyer flasks with 25 mL of filtered (glass fibre filters, 47 mm diameter, pore size 0.7 μm; Millipore APFF04700, Millipore Corp. Billerica, MA, USA) stream water from the corresponding site. The flasks were incubated for 48 h on a shaker (100 rpm) placed in a room at 15 °C and with 12 h light: 12 h dark photoperiod. The conidial suspensions were then poured into 50 mL Falcon tubes, the flasks rinsed twice with distilled water, the suspensions fixed with 2 mL 37% formalin, the sample volume adjusted with distilled water to 35 mL, and the tubes stored in the dark. The litter discs were oven-dried at 105 °C for 24 h and weighed (±0.1 mg) to determine discs DM.

When preparing the slides for counting and identifying the conidia, 150 μL of Triton X–100 were added to the suspensions and mixed with a magnetic stirring bar. An aliquot of the suspensions was then filtered (SMWP membrane filters, 5 μm pore size; Millipore Corp., Billerica, MA, USA) with gentle vacuum and the filters were stained with 0.05% cotton blue in 60% lactic acid. Slides were scanned under a microscope at 200×(Graça et al., 2005). Sporulation rates were expressed as number of conidia released per mg DM per day and aquatic hyphomycete species richness was expressed as number of species per sample.

2.5. Data analysis

Water variables were compared between sites by *t*-test. Initial litter chemical variables were compared among litter species by 1-way analysis of variance (ANOVA), followed by Tukey's test when

significant effects were detected in ANOVA. Correlations among initial litter chemical variables were assessed by Pearson correlation.

Decomposition rates (k, /d) of the six litter treatments (A, C, P, AC, AP and CP) and of alder [A(AC), A(AP)], chestnut [C(AC), C(CP)] and poplar [P(AP), P(CP)] leaves incubated in 2-species mixtures were estimated by linear regression of ln-transformed proportion of DM remaining (negative exponential model) considering the intercept at ln(1) = 0. Litter decomposition was compared between sites and the six litter treatments and between sites and litter species incubated individually and in 2-species mixtures for each litter species by analysis of covariance (ANCOVA; time as covariable) followed by Tukey's test when significant effects were detected in ANCOVA. Overall litter decomposition rates of single-species and mixture treatments were estimated as the average of the decomposition rates of the three single-species treatments (A, C and P) and of the three mixture treatments (AC, AP and CP), respectively. Litter decomposition rates were compared between sites and single-species and mixture treatments by 2-way ANOVA. The relationships between decomposition rates of the six litter treatments at each site and initial litter chemical variables were assessed by linear regression. Expected decomposition rates for mixture treatments were estimated as the average of decomposition rates of the component litter species. Expected and observed decomposition rates for litter mixtures were compared for each site by paired t-test. The ratios between expected and observed decomposition rates for mixtures were compared between sites R and N by paired t-test.

The nutrient enrichment effect was determined as the response ratio (R), given by the ratio between litter decomposition rate at site N and litter decomposition rate at site R ($R = k_{SiteN}/k_{SiteR}$); R = 1 indicates no effect of nutrient enrichment, R<1 indicates an inhibition and R>1 indicates a stimulation of litter decomposition at site N compared with site R. Significant effects occur when the 95% confidence limit (CL) does not include 1 ($p < 0.050$).

Sporulation rates and aquatic hyphomycete species richness were compared among sites and the three litter species incubated individually and among sites and litter species incubated individually and in 2-species mixtures for each litter species over time by 3-way ANOVAs, followed by Tukey's test when significant effects were detected in ANOVA.

Ordination of aquatic hyphomycete communities was done by non-metric multidimensional scaling (NMDS) based on Bray-Curtis similarity matrix of log(x+1) transformed data (conidial production). Communities were compared among sites and litter species incubated individually and in mixtures by analysis of similarity (ANOSIM)(Primer 6 v6.1.11 & PERMANOVA+ v1.0.1; Primer-E Ltd, Plymouth, UK; Clarke and Gorley, 2001).

Data normality and homoscedasticity were checked by the Shapiro-Wilk's and Bartlett chi-squared test, respectively. When one of these assumptions was not met, data were transformed before analyses (please see tables with statistical results in Appendix A). Data analyses were done using STATISTICA v7 software (Statsoft, Tulsa, OK, USA) unless indicated otherwise.

3. Results

3.1. Litter decomposition

Litter mass loss was generally fast across treatments for both sites (Fig. 1). Despite the tendency for faster decomposition rates (38–44%) in single-species than in mixture treatments (Fig. 1A), differences were not significant (2-way ANOVA, p = 0.139; Table A1). Nutrient enrichment stimulated litter decomposition by 23 (mixture) – 28% (single-species) (Fig. 2A), but differences between sites were not significant (2-way ANOVA, p = 0.306; Table A1).

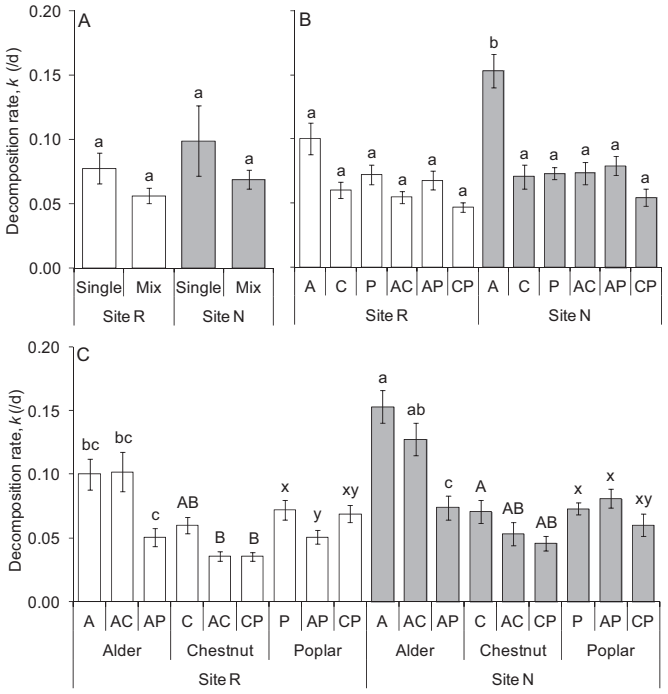

Fig. 1. Decomposition rates (mean ± SE) of (A) single-species and mixtures, (B) litter treatments (A, alder; C, chestnut; P, poplar; AC; AP; CP), and (C) litter species from single-species and mixture treatments incubated at sites R and N. Within each comparison, different letters indicate significant differences (Tukey's test, $p < 0.050$).

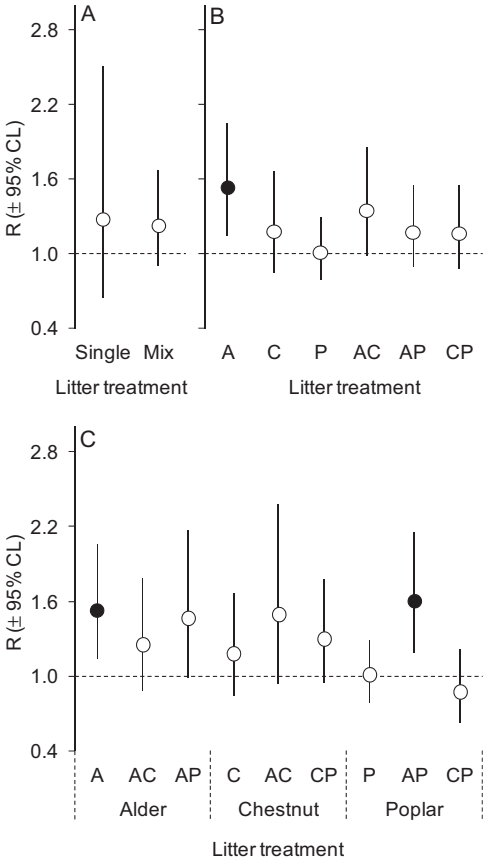

Fig. 2. Nutrient enrichment effect (R±95% CL) on decomposition rates of (A) single-species and mixtures, (B) litter treatments (A, alder; C, chestnut; P, poplar; AC; AP; CP), and (C) litter species from single-species and mixture treatments incubated at sites R and N. Significant effects ($p < 0.050$) are shown in black.

Table 3
Observed and expected decomposition rates ($k \pm$ SE) of litter mixtures incubated at sites R and N. The ratio between expected and observed (Exp/Obs) decomposition rates is also shown.

Site	Mixture	Observed k (/d)	Expected k (/d)	Exp/Obs
Site R	AC	0.0543 ± 0.0047	0.0775 ± 0.0202	1.43
	AP	0.0675 ± 0.0075	0.0851 ± 0.0140	1.26
	CP	0.0467 ± 0.0035	0.0658 ± 0.0062	1.41
Site N	AC	0.0732 ± 0.0089	0.1039 ± 0.0413	1.42
	AP	0.0791 ± 0.0071	0.1057 ± 0.0401	1.34
	CP	0.0543 ± 0.0066	0.0717 ± 0.0012	1.32

A, alder; C, chestnut; P, poplar.

Table 4
Linear regressions between litter decomposition rates (k,/d) of the six litter treatments incubated at sites R and N and initial litter chemical characteristics (n = 6). Sign, coefficients of determination (R^2) and p values are shown.

Litter variables	Site	Sign	R^2	p
Nitrogen (% DM)	Site R	+	0.41	0.172
	Site N	+	0.70	0.037
Phosphorus (% DM)	Site R	–	0.42	0.165
	Site N	–	0.27	0.294
Polyphenols (% DM)	Site R	–	0.27	0.289
	Site N	–	0.57	0.084
Lignin (% DM)	Site R	–	0.09	0.563
	Site N	–	0.32	0.242

Litter decomposition rates did not significantly differ among the six litter treatments at site R, but alder incubated individually decomposed significantly faster than any other litter treatment at site N (ANCOVA, p = 0.032; Fig. 1B, Table A1). Decomposition rates of litter mixtures were significantly slower than expected from the average of the decomposition rates from species incubated individually by 26–43% at site R (paired t-test, p = 0.007) and 32–42% at site N (p = 0.024), suggesting antagonistic effects of litter mixing (Fig. 1B; Table 3). Nutrient enrichment stimulated litter decomposition rates between 1 and 53% (average across treatments: 23%), but significant effects were found only for alder incubated individually (Fig. 2B, Table A1). The ratio between expected and observed decomposition rates for mixtures did not significantly change between sites R and N (paired t-test, p = 0.916; Table 3).

When comparisons were made within each species, alder decomposed slower when incubated together with poplar [A(AP)] than when incubated individually, although significant differences were found only at site N (Fig. 1C, Table A1). Chestnut litter tended to decompose slower when incubated with alder or poplar than when incubated individually (Fig. 1C, Table A1). Poplar decomposed slower when incubated together with alder [P(AP)] than when incubated individually at site R, while no significant effect of mixing was found at site N (Fig. 1C, Table A1). The effect of nutrient enrichment on the decomposition of individual litter species varied from inhibition by 13% (poplar in CP mixture) to stimulation by >30% (alder incubated individually and in AP mixture, chestnut in AC and CP mixtures and poplar in AP mixture)(Fig. 2C), although significant effects were found only in the case of the stimulated decomposition of alder incubated individually (stimulation by 53%) and poplar in AP mixture (stimulation by 60%)(Fig. 2C, Table A1).

Litter decomposition rates of the six litter treatments were significantly related with initial N concentration at site N (linear regression, p = 0.037) but not at site R (Table 4). No significant relationship was found between litter decomposition rates and phosphorus, phenols or lignin initial concentrations (Table 4). It is relevant to note that initial concentrations of N and phenols and of N and lignin were negatively correlated (Pearson correlation, r = –0.97, p < 0.001 and r = –0.83, p = 0.001, respectively) while those of phenols and lignin were positively correlated (r = 0.94, p < 0.001; Table A2).

3.2. Conidial production by aquatic hyphomycetes

Sporulation rates by aquatic hyphomycetes were already high by the first sampling date (day 7) and generally decreased thereafter (Fig. 3, Table A3). Sporulation rates did not significantly differ among the three litter species incubated individually (3-way ANOVA, p = 0.481) and between sites within each sampling date (3-way ANOVA, p = 0.586) (Table A3). When comparisons were made within each litter species, also no significant differences were found between litter treatments and sites for alder, chestnut and poplar (3-way ANOVA, p > 0.213; Fig. 3, Table A3).

Aquatic hyphomycete species richness generally increased until day 21, decreasing afterwards for chestnut and poplar (Fig. 3, Table A3). Species richness significantly differed among litter species incubated individually (alder >chestnut ~poplar; 3-way ANOVA, p = 0.003) and between sites (site N >site R; p = 0.038)(Table A3). When comparisons were made within each species, significant differences were found between sites N and R for alder, chestnut and poplar (3-way ANOVA, p < 0.022; Table A3) with higher richness at site N (Fig. 3).

The total number of aquatic hyphomycete species recorded per treatment varied between 11 and 16, but in most cases two species were enough to guarantee >80% of total conidial production. Conidial production in alder litter, incubated either individually or in mixtures, had a larger contribution of *Flagellospora curvula* (47.0–73.5% of total conidial abundance), followed by *Tetrachaetum elegans* (14.0–34.4%), and to a lesser extent by *Stenocladiella neglecta* (3.4–6.4%), *Tricladium chaetocladium* (3.1–5.4%) and *Clavariopsis aquatica* (0.9–5.3%)(Table A4). In chestnut, conidial production had a larger contribution of *T. elegans* (48.5–65.2%), followed by *T. chaetocladium* (14.0–20.2%) and *F. curvula* (10.2–19.9%), and to a lesser extent by *C. aquatica* (3.3–7.6%)(Table A4). In poplar, the species that most contributed to conidial production was *F. curvula* (45.3–70.0%), followed by *T. elegans* (15.0–30.1%) and *C. aquatica* (6.0–16.8%), and to a lesser extent *T. chaetocladium* (2.1–8.6%)(Table 8). Aquatic hyphomycete community structure significantly differed among litter species (ANOSIM, global R = 0.90, p = 0.010; Fig. 4). Within each litter treatment, species contribution to total conidial production tended to differ between sites R and N (Fig. 4); for instance, for alder *F. curvula* contribution increased while that of *T. elegans* decreased at site N (Table A4). However, overall differences in community structure between sites were not significant (ANOSIM, global R = 0.26, p = 0.065). Community structure within each litter species was also not significantly affected by mixing (ANOSIM, global R = –0.11, p = 0.732).

4. Discussion

In this study we assessed the effects of whole-stream N enrichment and litter species mixing on litter decomposition and associated fungi to better understand the response of stream ecosystems to simultaneous changes in nutrient availability and identity and number of species in the leaf litter.

Increases in nutrient availability in oligotrophic streams generally stimulate litter decomposition (Ferreira et al., 2015), but in our study this effect was significant only for alder incubated individually. This result was surprising for two reasons. First, the study stream was N limited and thus it was expected that an increase in N concentration would stimulate litter decomposition across treatments, as observed before (Ferreira et al., 2006). Also, a strong effect of nutrient enrichment on litter decomposition was expected due to the warmer water temperature during the study period,

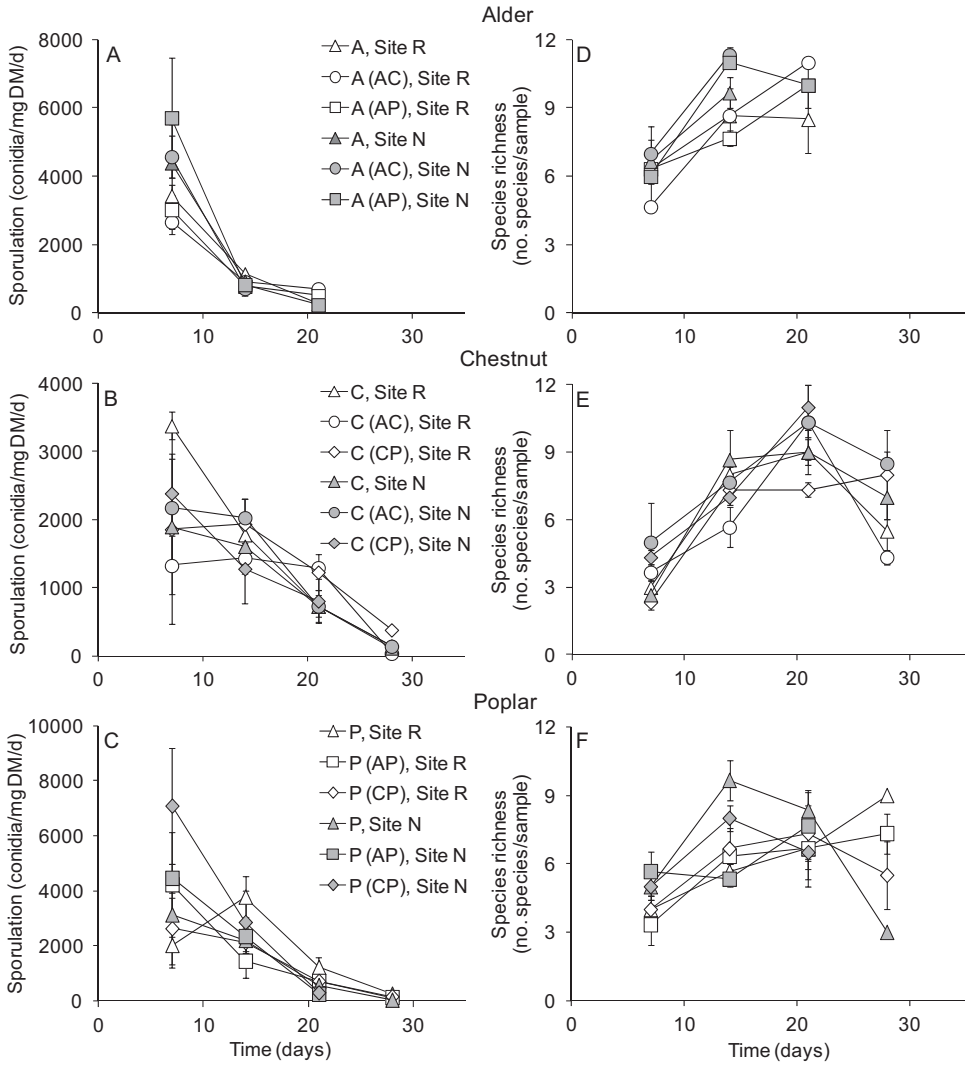

Fig. 3. Conidial production rate and species richness (mean ± SE) of aquatic hyphomycetes associated with alder (A; A and D), chestnut (C; B and E) and poplar (P; C and F) litter from single-species and mixture treatments (AC, AP, CP) incubated at sites R and N over time.

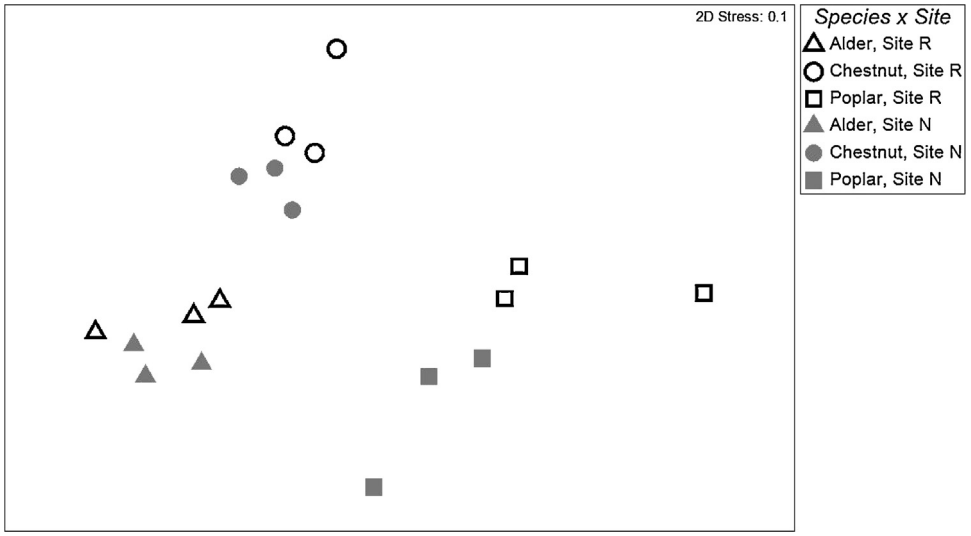

Fig. 4. Ordination (NMDS) of aquatic hyphomycete communities based on conidial production associated with alder, chestnut and poplar litter from single-species and mixture treatments incubated at sites R and N over time.

which could reinforce the nutrient effect (Ferreira and Chauvet, 2011). However, a recent study showed that lower N concentrations are needed for fungal decomposers to attain maximum activity at warmer temperature than at lower temperature (Fernandes et al., 2014) suggesting that decomposer activity may have been already at maximum at the reference site and thus not sensitive to nutrient enrichment. Second, increases in dissolved nutrients concentration generally have a stronger stimulatory effect on the decomposition of low quality litter species where microbial activity is nutrient limited than on high quality litter species (Gulis and Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006) and thus a stronger stimulation of litter decomposition was expected for chestnut and poplar than for alder litter. Our results, however, agree with those of Ardón et al. (2006), who found the strongest response of litter decomposition to increases in phosphorus concentration for the high quality litter species (*Tremma integerrima*, with the lowest concentration of structural compounds). In both our and Ardón et al. (2006) study, the quality of the litter carbon may have been more important than litter nutrient concentration in determining the response of microbial activity and litter decomposition to nutrient enrichment. N enrichment of forest floor has been found to stimulate cellulase (cellulose degrading enzyme) activity and to inhibit phenol oxidase (lignin degrading enzyme) activity (Carreiro et al., 2000). Lignin concentration was lower in alder (our study) and in *T. integerrima* (Ardón et al., 2006) than in the other litter species, and thus an inhibition of phenol oxidase activity by nutrient enrichment would have minor effects on litter decomposition for these species than for lignin richer species.

Litter decomposition is generally faster for high quality (low lignin and high nutrient concentration) than for low quality litter, as microbial colonization and activity are generally higher in the former (Gessner and Chauvet, 1994; Schindler and Gessner, 2009; Lima-Fernandes et al., 2015; Frainer et al., 2015). In our study, differences among litter treatments were found only at site N with faster decomposition for the nutrient rich alder litter than for the other five litter treatments. Also, only at site N was there a significant relationship between litter decomposition and initial litter N concentration. The absence of an effect of litter treatment (or initial litter chemical composition) on litter decomposition at site R is striking, especially considering the range in initial litter N (0.71–2.19%) and lignin concentration (28.61–46.47%) used. However, our results agree with a recent study showing that the dependence of litter decomposition on litter initial nitrogen concentration increased with eutrophication (Lima-Fernandes et al., 2015). As the availability of dissolved nutrients increases, microbial decomposers may become more efficient in using litter carbon. Also, enhanced litter quality at site N might have stimulated consumption by invertebrate detritivores, which might have exacerbated differences between leaf species.

Decomposition rates of all litter mixtures were lower than those expected from the decomposition rates of the component litter species incubated individually (k observed < k expected), at both sites, suggesting antagonistic effects of litter mixing. This partially agrees with our predictions as non-additive effects of litter mixing were expected (Lecerf et al., 2011). However, these effects were anticipated to occur only at site R, not at site N (Rosemond et al., 2010; Lima-Fernandes et al., 2015). Since microbes can take up nutrients from both the litter and the water (Suberkropp, 1991; Gulis and Suberkropp, 2003), an increase in dissolved nutrients concentration could make microbes less dependent on litter nutrients and thus the effects of litter mixing on microbial activities and litter decomposition were expected to become weaker at site N (Rosemond et al., 2010; Lima-Fernandes et al., 2015). However, this study was carried out in late spring-early summer, and nutrient enrichment likely interacted with temperature leading to no change in the ratio between expected and observed decomposition rates between site R and site N (Swan and Palmer, 2004).

Also, non-additive effects of litter mixing were expected primarily for litter mixtures composed of litter species with contrasting characteristics (AC and AP) as substrate heterogeneity and complementarity would be greater in these mixtures, which could promote biotic colonization and activity (Hansen and Coleman, 1998; Kominoski et al., 2009). However, the few studies addressing the existence of litter trait complementarity on the decomposition of litter mixtures have found weak evidence, at the most, for such effect on litter decomposition and fungal decomposers (Schindler and Gessner, 2009; Frainer et al., 2015). Our results are in line with these studies as non-additive effects of litter mixing were found for all three litter mixture and were not related with species composition.

To allow better understanding of the mechanisms that led to the observed non-additive effects of litter mixing on litter decomposition, component litter species were processed individually and decomposition and associated fungal reproductive activity were assessed. Litter in mixtures decomposed at similar or slower rates when compared with litter incubated individually. Slower decomposition of litter when in mixture with litter of lower quality [e.g. A(AC), A(AP) and C(CP)] than when incubated individually might be due to leaching of secondary compounds (e.g. polyphenols) from the lower quality litter that can inhibit microbial activity on the higher quality litter (Canhoto and Graça, 1999). Slower decomposition of litter when in mixture with litter of higher quality [e.g. C(AC) and P(AP)] could be due to selective feeding of detritivores on the higher quality litter.

Conidial production by aquatic hyphomycetes for each sampling date was not affected by nutrient enrichment, litter species or mixing. This was surprising since fungal reproductive activity is usually stimulated by increases in dissolved nutrient concentrations (Suberkropp and Chauvet, 1995; Gulis and Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006). Sporulation rates generally increase in the first days to weeks after litter submersion, attain a peak and then decrease at later stages of litter decomposition (Gessner and Chauvet, 1994; Suberkropp and Chauvet, 1995). In our study, however, we missed the increasing phase of the sporulation dynamics and by day 7 (first sampling date) sporulation rates were already at their peak or decreasing, which may have prevented us from detecting an effect of nutrient enrichment as sporulation rates may become more homogeneous across treatments at later stages of litter decomposition (Gulis et al., 2006). No effect of litter species was also unexpected as previous studies have shown higher sporulation rates on high quality than on low quality litter species (Gulis et al., 2006; Ferreira et al., 2012), but again, missing the raising phase to the sporulation dynamics may have prevented differences among treatments from being found.

We cannot, however, exclude the possibility that the nutrient enrichment (1.7 mg NO_3-N/L at site N) might have limited fungal reproductive activity. In fact, in a laboratorial experiment, Fernandes et al. (2014) found a unimodal relationship (explained by a Lorentzian model) between conidial production by aquatic hyphomycetes on alder leaf discs and nitrogen concentration (0.3–5.0 mg NO_3-N/L), with maximum sporulation rates at 2.97 mg NO_3-N/L at 12 °C and 1.07 mg NO_3-N/L at 18 °C. Although water temperature at ~9 am in our stream was close to 12 °C, maximum water temperature reached 15.8 °C (personal observation), at which the nutrient enrichment might have been inhibitory of reproductive activity. For low quality oak leaf discs, the relationship between sporulation rates and nitrogen concentration was, however, linear at 12 °C and asymptotic at 18 °C (Fernandes et al., 2014), and we could expect a higher sporulation rate at site N compared with site R for the more recalcitrant chestnut and poplar in our stream. Also, the higher aquatic hyphomycete species richness

per sampling date at site N than at site R might have led to intraspecific interactions possibly limiting conidial production. Reduction in conidial production in aquatic hyphomycete species mixtures when compared with fungal species in isolation were found before (Treton et al., 2004).

Aquatic hyphomycetes species richness for each sampling date was higher at the N enriched site than at the reference site and higher for the high quality alder litter than for chestnut and poplar, but no effect of mixing was found. Conditions with higher nutrient availability (e.g. stream nutrient enrichment or nutrient rich litter species) may support higher number of species at a given time due to a release of interspecific competition for resources, and this has been found before (Gulis and Suberkropp, 2003). Aquatic hyphomycetes communities were structured by litter identity and to a lesser extent by site. This result agrees with previous studies where distinct aquatic hyphomycetes communities have been found for different litter species (Canhoto and Graça, 1996; Gulis, 2001; Ferreira et al., 2006) and stream sites of contrasting nutrient concentration (Gulis and Suberkropp, 2003; Castela et al., 2008; Lima-Fernandes et al., 2015). This probably results from aquatic hyphomycetes species differing in enzymatic performance (Zemek et al., 1985; Chandrashekar and Kaveriappa, 1988) and nutrient concentration (Grimmett et al., 2013; Danger et al., 2016) and thus having distinct capabilities to decompose substrates and distinct nutrient requirements (Bisht, 2013). No effect of litter mixing was found on the community structure of aquatic hyphomycetes associated with each litter species suggesting that the identity of the neighboring litter species is of little importance.

In summary, our study produced several unexpected results, mostly related with the absence of a strong effect of nutrient enrichment and litter identity on fungal reproductive activity and litter decomposition. However, fungal reproductive activity and litter decomposition showed similar patterns and thus we are confident on our results. It is worthy to highlight that this study was performed in late spring-early summer, a time of the year less explored in terms of how detrital food webs work and how they respond to environmental change in temperate streams when compared with autumn-winter (but see Gessner et al., 1993; Nikolcheva and Bärlocher, 2005; Ferreira and Canhoto, 2015). In our study, litter decomposition rates were among the highest values reported for the same litter species (Abelho, 2001; Woodward et al., 2012), likely as a result of the warmer temperature that stimulated decomposer activity (Ferreira et al., 2014); sporulation rates were also among the highest values reported (Ferreira et al., 2012). Also, in late spring-early summer, litter benthic standing stock is reduced in streams flowing through native deciduous forests (Molinero and Pozo, 2004) and invertebrate detritivores, which are mostly insect larvae already in their late instars and thus near their maximum biomass, might have concentrated in the litter bags further stimulating litter decomposition (Frainer et al., 2014). Thus, the already high activity of microbial decomposer and detritivores in late spring-early summer might have masked potential effects of nutrient enrichment and litter identity on litter decomposition. We found, however, non-additive effects of litter mixing on litter decomposition that did not depend on dissolved nutrient concentration, suggesting that changes in riparian vegetation composition may have unpredictable effects on litter decomposition independently of the trophic state of streams.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.limno.2016.03.002.

Bibliography

APHA, 1995. Standard methods for the examination of water and wastewater. 19th ed. Eaton, A.E., Clesceri, L.S., Greenberg, A.E., (Eds.), Washington, D.C.
Abelho, M., Graça, M.A.S., 1998. Litter in a first-order stream of a temperate deciduous forest. Hydrobiologia 386, 147–152 (Margaraça Forest, central Portugal).
Abelho, M., 2001. From litterfall to breakdown in streams: a review. Sci. World J. 1, 656–680.
Allan, J.D., Castillo, M.M., 2007. Stream Ecology: Structure and Function of Running Waters. Springer Dordrecht, the Netherlands.
Ardón, M., Stallcup, L.A., Pringle, C.M., 2006. Does leaf quality mediate the stimulation of leaf breakdown by phosphorus in neotropical streams? Freshwater Biol. 51, 618–633.
Bisht, S., 2013. Growth responses of aquatic hyphomycetes to different sources of carbon and nitrogen. J. Appl. Nat. Sci. 5, 313–317.
Canhoto, C., Graça, M.A.S., 1996. Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. Hydrobiologia 333, 79–85.
Canhoto, C., Graça, M.A.S., 1999. Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. Microb. Ecol. 37, 163–172.
Canhoto, C., Graça, M.A.S., 2008. Interactions between fungi and stream invertebrates: back to the future. In: Sridhar, S., Bärlocher, F., Hyde, K.D. (Eds.), Novel Techniques and Ideas in Mycology Fungal Diversity. Hong Kong University, Research Series 20.
Canhoto, C., Gonçalves, A.L., Bärlocher, F., 2016. Biology and ecological functions of aquatic hyphomycetes in a warming climate. Fungal Ecol. 19, 201–218.
Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.
Castela, J., Ferreira, V., Graça, M.A.S., 2008. Evaluation of stream ecological integrity using litter decomposition and benthic invertebrates. Environ. Poll. 153, 440–449.
Chandrashekar, K.R., Kaveriappa, K.M., 1988. Production of extracellular enzymes by aquatic hyphomycetes. Folia Microbiol. 33, 55–58.
Clarke, K.R., Gorley, R.N., 2001. Primer v5: User Manual/Tutorial. Primer-E Ltd., Plymouth, UK.
Danger, M., Gessner, M.O., Bärlocher, F., 2016. Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. Fungal Ecol. 19, 100–111.
Fernandes, I., Seena, S., Pascoal, C., Cássio, F., 2014. Elevated temperature may intensify the positive effects of nutrients on microbial decomposition in streams. Freshwater Biol. 59, 2390–2399.
Ferreira, V., Canhoto, C., 2015. Future increase in temperature may stimulate litter decomposition in temperate mountain streams: evidence from a stream manipulation experiment. Freshwater Biol. 60, 881–892.
Ferreira, V., Chauvet, E., 2011. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Glob. Change Biol. 17, 551–564.
Ferreira, V., Voronina, E., 2016. Impact of climate change on aquatic hypho- and terrestrial macromycetes. In: Marxsen, J. (Ed.), Climate Change and Microbial Ecology: Current Research and Future Trends. Caister Academic Press, Norfolk, UK.
Ferreira, V., Gulis, V., Graça, M.A.S., 2006. Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia 149, 718–729.
Ferreira, V., Encalada, A.C., Graça, M.A.S., 2012. Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. Freshwater Sci. 31, 945–962.
Ferreira, V., Gulis, V., Pascoal, C., Graça, M.A.S., 2014. Stream pollution and fungi. In: Jones, G., Hyde, K., Pang, K.-L. (Eds.), Freshwater Fungi and Fungus-like Organisms. De Gruyter Series: Marine and Freshwater Botany. De GruyterBerlin, Germany, pp. 389–412 (Chapter 18).
Ferreira, V., Castagneyrol, B., Koricheva, J., Gulis, V., Chauvet, E., Graça, M.A.S., 2015. A meta-analysis of the effects of nutrient enrichment on litter decomposition in streams. Biol. Rev. 90, 669–688.

Frainer, A., McKie, B.G., Malmqvist, B., 2014. When does diversity matter? Species functional diversity and ecosystem functioning across habitats and seasons in a field experiment. J. Anim. Ecol. 83, 460–469.
Frainer, A., Moretti, M.S., Xu, W., Gessner, M.O., 2015. No evidence for leaf-trait dissimilarity effects on litter decomposition, fungal decomposers, and nutrient dynamics. Ecology 96, 550–561.
Gessner, M.O., Chauvet, E., 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75, 1807–1817.
Gessner, M.O., Thomas, M., Jean-Louis, A.M., Chauvet, E., 1993. Stable successional patterns of aquatic hyphomycetes on leaves decaying in a summer cool stream. Mycol. Res. 97, 163–172.
Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends Ecol. Evol. 25, 372–380.
Goering, H.K., Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). USDA Agricultural Handbook No. 379.
Graça, M.A.S., Pozo, J., Canhoto, C., Elosegi, A., 2002. Effects of eucalyptus plantations on detritus, decomposers, and detritivores in streams. Sci. World J. 2, 1173–1185.
Graça, M.A.S., Bärlocher, F., Gessner, M.O., 2005. Methods to Study Litter Decomposition. A Practical Guide. Springer Dordrecht, the Netherlands.
Grimmett, I.J., Shipp, K.N., Macneil, A., Bärlocher, F., 2013. Does the growth rate hypothesis apply to aquatic hyphomycetes? Fungal Ecol. 6, 493–500.
Gulis, V., Suberkropp, K., 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biol. 48, 123–134.
Gulis, V., Ferreira, V., Graça, M.A.S., 2006. Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshwater Biol. 51, 1655–1669.
Gulis, V., 2001. Are there any substrate preferences in aquatic hyphomycetes? Mycol. Res. 105, 1088–1093.
Hansen, R.A., Coleman, D.C., 1998. Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari: oribatida) in litterbags. Appl. Soil Ecol. 9, 17–23.
Kominoski, J.S., Hoellein, T.J., Kelly, J.J., Pringle, C.M., 2009. Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? Oikos 118, 457–463.
Kominoski, J.S., Shah, J.J.F., Canhoto, C., Fischer, D.G., Giling, D.P., Gonzalez, E., Griffiths, N.A., Larrañaga, A., LeRoy, C.J., Mineau, M.M., McElarney, Y.R., Shirley, S.M., Swan, C.M., Tiegs, S.D., 2013. Forecasting functional implications of global changes in riparian plant communities. Front. Ecol. Environ. 11, 423–432.
Lecerf, A., Chauvet, E., 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic Appl. Ecol. 9, 598–605.
Lecerf, A., Marie, G., Kominoski, J.S., LeRoy, C.J., Bernadet, C., Swan, C.M., 2011. Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition. Ecology 92, 160–169.
Lima-Fernandes, E., Fernandes, I., Pereira, A., Geraldes, P., Cássio, F., Pascoal, C., 2015. Eutrophication modulates plant-litter diversity effects on litter decomposition in streams. Freshwater Sci. 3, 31–41.
MEA (Millennium Ecosystem Assessment), 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC.
Molinero, J., Pozo, J., 2004. Impact of a eucalyptus (*Eucalyptus globulus* Labill.) plantation on the nutrient content and dynamics of coarse particulate organic matter (CPOM) in a small stream. Hydrobiologia 528, 143–165.
Molinero, J., Pozo, J., 2006. Organic matter, nitrogen and phosphorus fluxes associated with leaf litter in two small streams with different riparian vegetation: a budget approach. Arch. Hydrobiol. 166, 363–385.
Mulholand, P.J., Steinman, A.D., Marzolf, E.R., Hart, D.R., DeAngelis, D.L., 1994. Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. Oecologia 98, 40–47.
Nikolcheva, L.G., Bärlocher, F., 2005. Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. Environ. Microbiol. 7, 270–280.
Paiva, J.A.R., 1981. Mata da Margaraça e sua conversão em reserva. Ann. Soc. Brot. 47, 49–66.
Rockström, J., Steffen, W., Noone, K., Persson, A., Chapin III, F.S., Lambin, E., Lenton, T.M., Scheffer, M., Folke, C., Schellnhuber, H.J., Nykvist, B., de Wit, C.A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley, J., 2009. Planetary boundaries: exploring the safe operating space for humanity. Nature 461, 472–475.
Rosemond, A.D., Swan, C.M., Kominoski, J.S., Dye, S.E., 2010. Non-additive effects of litter mixing are suppressed in a nutrient-enriched stream. Oikos 119, 326–336.
Schindler, M.H., Gessner, M.O., 2009. Functional leaf traits and biodiversity effects on litter decomposition in a stream. Ecology 90, 1641–1649.
Suberkropp, K., Chauvet, E., 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76, 1433–1445.
Suberkropp, K., 1991. Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. Mycol. Res. 95, 843–850.
Swan, C.M., Palmer, M.A., 2004. Leaf diversity alters litter breakdown in a Piedmont stream. J. N. Am. Benthol. Soc. 23, 15–28.
Treton, C., Chauvet, E., Charcosset, J.Y., 2004. Competitive interaction between two aquatic hyphomycete species and increase in leaf litter breakdown. Microb. Ecol. 48, 439–446.
Wallace, J.B., Eggert, S.L., Meyer, J.L., Webster, J.R., 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277, 102–104.
Webster, J.R., Ehrman, T.P., 1996. Solute dynamics. In: Hauer, F.R., Lamberti, G.A. (Eds.), Methods in Stream Ecology. Academic Press, San Diego, CA, pp. 145–160.
Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graça, M.A.S., Fleituch, T., Lacoursiere, J., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.-M., Chauvet, E., 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336, 1438–1440.
Zemek, J., Marvanová, L., Kuniak, Ł., Kadlečíková, B., 1985. Hydrolytic enzymes in aquatic hyphomycetes. Folia Microbiol. 30, 363–372.